Introduction of an IAS should not adversely affect (i) sequence-specific binding of the template to the modified primer, (ii) sequence-specific binding of the primer to the complementary oligonucleotide **83**, (iii) enzymatic extension of the primer, or (iv) the cutting ability of the restriction enzyme. Generally, the extension product is immobilized and washed to remove reaction products (salts, enzymes, nucleotide fragments, reagents) prior to release and subsequent size and or sequence analysis. Other approaches include (i) the use of a primer or extension segment containing an immobilization attachment site, where, after enzymatically extending the primer and denaturing the double stranded product, the single stranded primer-extension product is captured *via* binding at the immobilization attachment site, followed by removal of the template and addition of complementary oligonucleotide **83**, as described above, or (ii) the use of a template modified to contain an immobilization attachment site, for capturing the template either prior to or after enzymatic extension, prior to addition of oligonucleotide **83**.

A variation of a cleavable primer of the type illustrated in FIG. 11 is shown in FIG. 16, where the first primer region contains a universal restriction recognition site within a hairpin (Szybalski, 1985). Referring now to FIG. 16, the cleavable site **127** is a class IIs restriction endonuclease cleavable site, where the double stranded enzyme recognition sequence **129** is located in the first primer region (*i.e.* upstream of the cleavage site), and the first primer region contains a 5′ hairpin-type (self-complementary double stranded) domain. The 5′ hairpin domain **131** includes the double stranded recognition site **129** for the restriction enzyme. The second (single stranded) primer region contains (i) the cleavable site (*i.e.* restriction endonuclease cut site), and (ii) is composed of nucleotides complementary to a single stranded target **133**, thus serving as a priming site for enzymatic extension. Following enzymatic extension of the primer (shown at **135**), the product **137** is cleaved **139** by treatment with a suitable class IIs restriction endonuclease to release fragments **141** and **143**, followed by denaturation to release the single stranded extension segment for subsequent analysis, *i.e.* by mass spectrometry. As indicated in the figure at **145**, the template may optionally be attached to a solid phase support at any stage during the process.

In some instances, the cleavable site is a nucleotide capable of blocking or terminating 5′ to 3′ enzyme-promoted digestion by an enzyme having 5′ to 3′ exonuclease activity, such as